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Acute and Subsequent Continuation Electroconvulsive Therapy Elevates Serum BDNF Levels in Patients with Major Depression

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ABSTRACT

Introduction: The induction of brain-derived neurotrophic factor (BDNF) release and subsequent restoration of neuroplastic homeostasis may underlie the effects of electroconvulsive therapy (ECT).

Objectives: We aimed to assess serum and plasma BDNF levels during the course of acute ECT, as well as before and after subsequent continuation ECT, in patients with depression.

Methods: We included 24 patients with major depressive disorder (mean age ± SD: 54.5±13.7; f/m: 17/7; baseline Hamilton Depression Rating Scale score: 17 of 26.79±4.01). Serum and plasma BDNF (sBDNF, pBDNF) levels were assessed at nine time-points before, during, and after acute ECT series. Data were analysed using linear regression and linear mixed models, which were adjusted for multiple comparisons via Bonferroni correction. Because they exhibited a good response to the acute ECT series, five patients underwent continuation ECT. In these patients, BDNF levels were assessed before and after two continuation ECT sessions using Wilcoxon signed-rank tests.

Results: Relative to baseline (mean ng/ml ±SD: 24.68 ±14.40), sBDNF levels were significantly higher 1 day (33.04 ±14.11, p=0.013, corrected), 1 week (37.03 ±10.29, p<0.001, corrected), and 1 month (41.05 ±10.67, p=0.008, corrected) after the final ECT session, while pBDNF levels did not significantly differ (p>0.1). Furthermore, our results indicated that sBDNF levels increased after each continuation ECT session. There was no significant association between sBDNF levels and clinical parameters or treatment response.

Conclusion: The absence of an association between changes in sBDNF levels and depressive symptoms challenges the proposed concept of sBDNF/pBDNF as key markers of the effects of ECT.

Keywords: electroconvulsive therapy, ECT, brain-derived neurotrophic factor, BDNF, major depressive disorder

INTRODUCTION[1]

Major depressive disorder (MDD) is among the most common medical illnesses worldwide, with a median 12-month prevalence rate of 6.9% [1]. Furthermore, MDD leads to individual harm [2] and socioeconomic strain [3]. Evidence suggests that psychopharmacological and non-psychopharmacological therapies should be considered for patients who fulfil criteria for MDD, with the aim of achieving remission [4].

Indications for electroconvulsive therapy (ECT) in patients with neuropsychiatric disorders are broad, and ECT can be used as an augmentative form of therapy at any time during the therapeutic process [4]. Research has demonstrated that ECT is the most effective treatment for MDD [5, 6]. Despite the long history and proven efficacy of ECT, the neurobiological mechanisms underlying its effects remain unclear [7, 8]. Preclinical and clinical data point towards morphological brain alterations [9, 10] as well as dysfunctional monoaminergic neurotransmission, especially that involving the modulation of 5-HT_{1A} and 5-HT_{2A} receptors. These findings are in accordance with the proposed mechanism of action for common pharmacological antidepressants [11, 12]. Furthermore, numerous studies have demonstrated that neurotrophic factors play a role in depression and other psychiatric disorders [13-15]. Thus, some reports have indicated that stress-associated alterations in neurotrophic factor signaling may underlie the development of mood disorders, while upregulation of neurotrophic factors has been linked to treatment responses [16, 17].

Most studies investigating neurotrophins in patients with psychiatric disorders have examined the role of brain-derived neurotrophic factor (BDNF) [18]. BDNF mediates numerous critical functions within the brain and is involved in neuronal maturation, synapse formation, and synaptic plasticity [19]. Post

[1] Abbreviations: MDD: major depressive disorder; ECT: electroconvulsive therapy; BDNF: brain-derived neurotrophic factor; sBDNF: serum BDNF; pBDNF: plasma BDNF; a-ECT: acute ECT; c-ECT: continuation ECT; HAMD-17: 17-item Hamilton Depression Rating Scale; BPRS: Brief Psychiatric Rating Scale; ELISA: enzyme-linked immunosorbent assay; CV: coefficient of variation; SCID: Structured Clinical Interview for the DSM-IV; BDI: Beck Depression Inventory; MMSE: Mini Mental State Examination; EEG: electroencephalography; EMG: electromyography; ECS: electroconvulsive stimulation.

mortem studies have revealed that patients with MDD and those who have committed suicide exhibit reduced levels of BDNF [20, 21]. In addition, prospective interventional studies have demonstrated that both antidepressant therapy and ECT increase peripheral BDNF levels in patients with MDD [19]. However, studies investigating BDNF levels before and after an ECT series in patients with unipolar and bipolar depression have yielded inconclusive results. Discrepancies in these findings are suggested to be caused by differences in applied methods, including the quantification of BDNF in serum or plasma, prescribed psychopharmacological treatment before and during ECT, the time-point of blood withdrawal after the last ECT, and differences in standard operating procedures for ECT [22]. Meta-analyses have reported that both serum and plasma BDNF (sBDNF, pBDNF) levels increase following an ECT series [18, 23, 24]. Since blood platelets (i.e., thrombocytes) contain a high amount of BDNF, sBDNF concentration is approximately 1,000 times higher than pBDNF concentration. Although previous studies have revealed associations between sBDNF and pBNDF levels in healthy participants [25], most ECT studies have investigated sBDNF levels only. Indeed, sBDNF levels are easier to detect, as they are more stable, more reliable, less prone to artifacts, and are present at higher concentrations than pBDNF. Some researchers have proposed methods for enhancing the quality of sBDNF assessment [26]. In addition, sBDNF has been identified as a reasonable proxy for brain BDNF levels, since serum and cortical BDNF levels exhibit an association with one another [27].

Although previous studies have assessed BDNF levels before and directly after acute ECT (a-ECT) series, knowledge regarding the time sequence of ECT-induced changes in BDNF levels remains lacking. No studies have investigated changes in BDNF levels during a-ECT (between first and last ECT), and only a few have investigated BDNF levels at several time-points after ECT. Subsequent to a-ECT, some patients undergo continuation ECT (c-ECT) approximately once per month to prevent relapse of depressive symptoms. Evidence from clinical studies is sparse, although c-ECT has been proven very effective in randomized controlled trials [28, 29]. Few studies have investigated the mechanisms by which c-ECT prevents relapse, and our understanding of the neurophysiological processes associated with c-ECT is

limited. Therefore, in the present study, we aimed to assess the time course of BDNF levels before, during, and after an a-ECT series, as well as before and after c-ECT, in order to expand our knowledge regarding the time-course of ECT-provoked changes in BDNF levels. We hypothesized that ECT would be associated with an increase in BDNF levels during the course of a-ECT and c-ECT.

METHODS

Study Participants and Clinical Assessment

Altogether, we included 31 adult treatment resistant depressive patients after insufficient response to previous treatment trials with antidepressants of different pharmacological classes or add on therapy with antipsychotics or mood stabilizers. BDNF levels and clinical data of 24 patients with major depressive disorder were analysed. Missing data subsequently to final ECT was caused by already discharged patients and were accounted for in statistical analyses (Table 3 shows the numbers of patients (n) per visit for various measures). Missing data subsequently to final ECT was caused by already discharged patients and were accounted for in statistical analyses. Twenty-one patients suffered from unipolar without psychotic features (ICD-10: F33.2) and 3 patients with psychotic features (ICD-10: F33.3). Based on retrospective assessments of recorded data, criteria for diagnosis of major depressive disorder according to DSM IV and DSM 5 were fulfilled by all patients included. We excluded two patients from analyses, since they developed a somatic disorder during the ECT. Further, due to the potential source of variability, we retrospectively excluded five patients with bipolar disorder. Participants were recruited at the Department of Psychiatry and Psychotherapy of the Medical University of Vienna. The Structured Clinical Interview for DSM-IV (SCID), the 17-item Hamilton Depression Rating Scale (HAMD-17) and the Brief Psychiatric Rating Scale (BPRS) was performed by senior psychiatrists to determine diagnosis and estimate illness severity. The study was approved by the Ethics Committee of the Medical University of Vienna and the General Hospital of Vienna (EC-number: 975/2010).

For eligibility to participate in the study patients had to fulfill inclusion criteria for ECT, including clinical approval for ECT as well as exclusion of any major somatic or neurological illness. All patients provided written informed consent for the study after detailed explanation of the procedure by an experienced psychiatrist. Concomitant psychopharmacological treatment in terms of antidepressants, antipsychotics and mood-stabilizing medication were kept in steady-state during the course of a-ECT. Within the course of the a-ECT series and up to one week post final a-ECT, a dose deviation in the range of 50% within one substance was tolerated. Benzodiazepines were administered as needed and paused before ECT. A part of the study sample received c-ECT once a month subsequent to the a-ECT series, where further sBDNF and pBDNF levels where investigated before and after two c-ECT.

Patients with major depressive disorder were included if the HAMD-17 total score was greater than or equal to 22. A 50 % reduction after the last ECT was defined as treatment response and a final (at V6) HAMD-17 score below seven defined remission. Next to the HAMD-17 and the BPRS, the test battery included the Beck Depression Inventar (BDI) for self-assessment of depressive symptoms and the Minimental State Examination (MMSE) to measure cognitive impairment.

Study Design

Longitudinal blood samples of sBDNF and pBDNF levels were investigated during the a-ECT period at a total of 9 visits (V): V1 and V2 were baseline visits two days and one day before the first ECT treatment; V3 was after the first ECT on the day of the first ECT; V4 was on the day after the first ECT; V5 was on the day after the 5^{th} ECT; V6 was on the day after the final ECT; V7 one week after the final ECT; V8 one month after the final ECT and V9 six months after the final ECT (Figure.1). We performed blood-withdrawal and subsequent processing from morning hours till noon (except for V3). For patients receiving c-ECT sBDNF and pBDNF levels were assessed before and after each c-ECT, while not being further included in analysis at V8 or V9 (except for one patient before c-ECT). In case of clinical feasibility, sBDNF and pBDNF were assessed twice instead of once before and after c-ECT (a day before

c-ECT; the day of c-ECT, before c-ECT; up to two hours after the c-ECT; the day after c-ECT). Clinical scores were assessed repetitively during the ECT series at V1, V5-V9 and before and after each c-ECT.

Electroconvulsive therapy

ECT was conducted using the Thymatrons® System IV device (Somatics, LLC, Lake Bluff, IL, USA) according to the Standard Operating Procedures (SOP) of the Department of Psychiatry and Psychotherapy that are based upon Frey et al 2001 [30] in concordance with guidelines and consensus statements for ECT [31]. The patients were anaesthesized with methohexital (about 10 mg per 10 kg body weight) In addition the muscle relaxans succinylcholine (about 10 mg per 10 kg body weight) was administered before the ECT stimulus. ECT was carried out three times a week. Five ECTs were provided unilaterally using electrode placements in the right frontotemporal and right parietal position. The stimulus intensity was chosen according to the titration at the first treatment [32], where repeated stimuli of increasing intensity were administered until a seizure occurred. Titration started with 50 mC (10% of maximum charge). The lowest stimulus intensity able to induce a seizure (mainly with 50 milliCoulomb, mC = 10%) was defined as the threshold. In the following treatments the charge was set as three times the seizure threshold. During the ECT course, the stimulus intensity was further elevated up to 1000 mC (= 200%) in case of inadequate seizures or in case of low efficacy. For patients with a lack of improvement a bilateral treatment (bifrontotemporal) approach was used from the sixth ECT session onward. The criteria for switching from unilateral to bilateral ECT were a HAMD score still $\geqslant$ 18. In case of an at least minor improvement under unilateral treatment with long seizure duration or memory deficits, clinical judgment could state for further high dose unilateral treatments. Bilateral treatment was initiated with the same dosage like the fifth unilateral treatment and elevated up to 1000 mC (= 200%) in case of inadequate seizures or in case of low efficacy. Seizure activity and duration was determined routinely by Thymatrons electroencephalography (EEG) and electromyogram (EMG). To assess seizure quality, we determined concordance as a parameter reflecting seizure inhibition [33],

which is motor seizure activity duration divided by EEG seizure activity duration, i.e. the ratio of EMG to EEG.

Sampling and assessment of serum BDNF

For the assessment of the BDNF level a blood sample was drawn using serum vacutainer tubes (Becton Dickinson) and a sodium citrate tube. We performed blood-withdrawal and subsequent processing from morning hours till noon (except for V3). The plasma tube was centrifuged at 1500xg for 15 minutes. After 30 minutes (room temperature) the serum tube was centrifuged at 1500xg for 15 minutes. Samples were stored at −80°C before analysis of BDNF levels. Serum BDNF levels were assessed with an enzyme-linked immunosorbent assay (ELISA) kit (Biosensis® Mature BDNF Rapid™ ELISA Kit: Human, Mouse, Rat; Thebarton, SA, Australia). Serum and plasma samples were appropriately diluted (1:100 for serum samples and 1:10, 1:5 and in some cases 1:25 for plasma samples) and detection of BDNF was carried out on a pre-coated mouse monoclonal anti-mature BDNF 96-well plate as described in the manufacturer's protocol. The absorbance was measured within 5 minutes after addition of the stop solution in a microplate reader set at 450nm and a correction wavelength set to 690nm, to determine BDNF concentrations according to the standard curve. All assays were carried out in duplicate and means were calculated. Intrinsic assay quality was evaluated by testing the intra-assay and the inter-assay coefficients of variation (CV). Intra-assay CV was assessed by comparing BDNF values measured twice into the same plate. Intra-assay CV (n=40) was ≤ 1% indicating very reproducible results. The same internal serum control samples (n=2) were run in duplicates on fourteen different plates as well as different days together with the patient samples to monitor plate-to-plate variation. The plate means for both internal controls were calculated and then used to calculate the overall mean, standard deviation, and % CV. Inter-assay CV (n=14) was 8.91%. Our assay quality values are in line with the declared ranges and findings from Polacchini et al. [26].

Statistical Analysis

We performed linear mixed models analyses to assess the effects of a-ECT on HAMD and BDI scores as well as on BDNF levels using visit (8 levels) and repeated samples at baseline (2 levels) as fixed factors and subject as the random factor. Post-hoc pairwise comparisons were corrected using the Bonferroni procedure. To test for potential confounders, separate interaction analyses were applied between visit and the covariates age, sex, diagnosis (depression with or without psychotic features), ECT stimulation mode (unilateral vs. bilateral), number of a-ECTs, treatment outcome (remitters, responders or non-responders; only for BDNF changes), concomitant medication (changes during a-ECT in the range of 50% within one substance or no changes), mean seizure duration (per patients per session) and seizure concordance (ratio between EMG and EEG separately). Similarly, a linear mixed model was used to test the effects of a-ECT on BDI and BPRS. In addition, regression analyses were performed to investigate the predictive power of baseline sBDNF values on HAMD and BDI changes after ECT treatment. A spearman rank correlation was applied to test for an association between baseline sBDNF values and BPRS. Here, baseline BDNF level was defined as the mean value of the first two BDNF assessments. Moreover, changes in BDNF were correlated with changes in HAMD over time. Nonparametric tests were applied for pBDNF, BPRS and MMSE values given violations of normal distribution. A Wilcoxon signed-rank test was performed on clinical scores and serum and plasma BDNF values in order to investigate changes before and after the two c-ECTs in seven patients, while mean sBDNF and pBDNF values were used in the case of double assessment before (a day before c-ECT; the day of c-ECT, before c-ECT) or after (the day of c-ECT, after c-ECT; the day after c-ECT) c-ECT. Moreover, we compared baseline sBDNF levels, HAMD-17 and BDI of the 5 patients receiving c-ECT and 19 patients that did not receive c-ECT by applying a two-sided t-test. The significance level was set at 5% in all analyses. The Bonferroni procedure was applied to correct post-hoc multiple comparisons for the main hypothesis of this paper (changes in BDNF levels over time during a-ECT). Secondary analyses including the test for covariates were exploratory in nature and p-values are provided at an uncorrected level of $p<0.05$. Statistical analysis was performed using IBM® SPSS® Statistics (Version 24).

RESULTS

Sample description and clinical endpoints

We included 24 patients with recurrent and severe depression (mean age ± SD: 54.5±13.7; f/m: 17/7; HAMD-17 baseline: 26.79±4.01). Patients received between 6 and 13 a-ECT, with a mean number of 9.6 (SD: ±1.7) ECTs. The patients mean cumulative seizure duration across the a-ECT series was 419.3 seconds (SD: ±148.1) in EEG and 288.9 seconds (SD: ±118.1) in EMG, the mean seizure duration for each seizure was 43.4 seconds (SD: ±13.74) in EEG and 30.1 seconds (SD: ±12.1) in EMG. In all 24 patients, we started with unilateral ECT. We started ECT with a right unilateral stimulation mode in all 24 patients. Due to an ineffective treatment after five ECT, we switched 8 patients to bilateral stimulation. According to HAMD-17 at V6, treatment outcome was divided into three groups: 7 remitters, 7 responders and 10 non-responders (see Table 1 for epidemiological, ECT specific information and treatment response to ECT). Concomitant medication was modified in 8 patients in the course of ECT (Table 2). Five out of 24 patients who received a-ECT subsequently received two c-ECT. Of these 5 patients, 2 remitted and 1 responded to the c-ECT, while two patients did not respond according to the criteria mentioned in the methods section. Nevertheless, these two patients reported relevant clinical improvement by ECT, which is evidenced in a reduction of HAMD-17 of 40% and 37%, respectively.

When evaluating treatment response by means of HAMD linear mixed models analyses revealed a main effect of visit (HAMD: $F=26.10$, $p<0.001$), with post-hoc t-tests showing significant symptom reduction at all visits compared to baseline (all $p<0.001$; Bonferroni corrected; see Table.3 and Figure.2). Significant symptom score reductions were also found using BDI and BPRS. No significant worsening of cognitive performance was detected using the MMSE (Table.3). Age, sex, diagnosis, number of ECTs, stimulation mode, concomitant medication, mean seizure duration (evaluated with EEG and EMG) and concordance had no effect on HAMD changes over time (no significant interactions with visit or main

effect of covariate).

BDNF changes over the course of acute ECT series

At baseline, mean sBDNF concentration was 24.68 ng/ml (SD ±14.4) while mean pBDNF concentration was 0.76 pg/ml (±1.46). Linear mixed model analysis revealed a main effect of visit ($F=4.51$, $p<0.001$) but no difference between the two baseline samples (main effect of repeated baseline samples, $F=0.35$, $p>0.1$). Post-hoc pairwise comparisons revealed a transient increase in sBDNF over time starting with significant differences to baseline at V6 ($p=0.013$), V7 ($p<0.001$) and V8 ($p=0.008$), but not at V9 ($p>0.1$; see Figure.3 and Table.1). Age, sex, treatment outcome, ECT stimulation mode, number of ECT, concomitant medication, mean seizure duration (measured with EMG) and concordance had no effects on sBDNF changes over time (all $p>0.10$; no significant interactions with visit or main effect of covariate). However, seizure duration assessed with EEG had a significant main effect on sBDNF ($F= 4.31$, $p=0.045$), but showed no interaction with time. Moreover, seizure duration did not affect the significant changes over time at V6, V7 and V8 (all $p>0.05$) when included as a covariate. Investigating pBDNF changes over time using non-parametric Friedman's ANOVA by ranks revealed no significant results ($p>0.1$).

Predicting treatment response by BDNF

Analysis revealed no effect of baseline sBDNF values on post-treatment HAMD (V6-V9; all $p>0.1$), BDI and BPRS (V6-V8; all $p>0.1$; a correlation with V9 was not computable). Moreover, baseline sBDNF values had no effect on the magnitude of HAMD reductions for any post-ECT visit (all $p>0.1$). Finally, associating sBDNF changes with changes in HAMD scores for any post-ECT visit compared to the baseline visit using Pearson correlation revealed no significant result (all $p>0.1$). Serum BDNF concentrations (ng/ml) before and after a-ECT are stated according to clinical outcome in Table.4.

Effects of continuation ECT

Investigating the effects of monthly c-ECT in the subsample of 5 patients on sBDNF levels using Wilcoxon signed-rank test revealed a significant increase after the first c-ECT (mean ng/ml ±SD; pre 1.ECT: 29.75 ±10.2, post 1.ECT: 57.2 ±14.4; p=0.043) and a non-significant increase after the second c-ECT session (pre 2.ECT: 30.67 ±14.1, post 2.ECT: 48.3 ±18.8; p<0.05; Figure.4). We found no significant differences in monthly pBDNF after c-ECT. Wilcoxon signed-rank test for clinical variables (HAMD-17, BPRS, BDI) revealed no significant pre-post changes after the first or second c-ECT. When comparing baseline (before a-ECT) sBDNF levels, HAMD-17 and BDI, we found trend-wise higher sBDNF concentrations for patients receiving c-ECT (31.63 ±8.0) compared to patients that only received a-ECT (21.58 ±13.22; p=0.056; Figure 4).

DISCUSSION

In the present study, we aimed to investigate the time-course of the effect of ECT on BDNF levels in patients with MDD. We observed significant increases in sBDNF levels during the course of a-ECT, which were followed by increases after c-ECT. Peak sBDNF levels were achieved 1 month following a-ECT. However, we observed no association between the antidepressant effect of ECT and increases in sBDNF levels. Furthermore, pBDNF levels remained unchanged during the course of a-ECT. Demographic and clinical factors such as diagnosis, stimulation mode, number of ECT session, outcome, concomitant medication, and ictal concordance exerted no significant influence on changes in sBDNF levels.

In accordance with the findings of human and animal studies, which have reported an association between latency and elevated BDNF levels after therapeutic convulsive stimulation [33-37], we observed steady increases in sBDNF levels beginning after the fifth ECT session. Peak sBDNF concentrations were achieved 1 month after the final a-ECT session. Also, we examined peak sBDNF concentrations following an acute ECT series and subsequent monthly continuation treatments (see Fig. 4). Evidence from animal studies has suggested that infusion of BDNF into the dentate gyrus, a subfield of the hippocampal complex, induces neurogenesis [38]. Furthermore, electroconvulsive

stimulation (ECS) has been shown to augment BDNF transcription in the cortex [39] and spark neurogenesis in the dentate gyrus [40]. In such studies, the greatest increases in cell proliferation were observed after five ECS sessions combined with subsequent c-ECS, although no such increases were observed after single or multiple ECS sessions [40]. In contrast to Bumb and colleagues, who reported a correlation between seizure quality and sBDNF levels in humans following a-ECT, we observed no influence of seizure markers (i.e., seizure concordance and duration) on sBDNF levels [33]. In conjunction with findings from animal studies, our results suggest that a certain number of ECT sessions is required to amplify the BDNF signaling pathway.

Critically, there is some question concerning the validity of measuring peripheral BDNF levels, which may limit the clinical application of our findings. Molecular and preclinical investigations have reported that BDNF is generated and stored in megakaryocytes and later in platelets [41], and that it does not pass the brain-blood barrier [42]. However, another study reported that BDNF can gradually pass through the blood-brain barrier [43]. Several studies have also reported positive and negative correlations between brain and sBDNF concentrations [27, 37, 44]. As the highest sBDNF levels are observed following multiple ECS sessions [33, 37], steady increases in blood levels of BDNF may be caused by BDNF gradually crossing the blood-brain barrier [33]. Nevertheless, brain BDNF levels have only been examined in animal studies and there is a lack of comparative tissue studies. Human clinical trials focused on circulating (blood) BDNF levels and other investigations regarding the relationship between brain and blood levels of BDNF have yielded inconclusive results. Thus, it remains unclear whether brain and peripheral levels of BDNF follow the same or dissimilar trajectories.

In the subgroup of patients who received monthly c-ECT (Fig. 4, left side), sBDNF levels were increased 1 week after the final a-ECT session and after each c-ECT session. In addition, baseline sBDNF concentrations tended to be higher in these five patients than in the remaining patients who underwent a-ECT only, while no differences in psychometric scores (HAMD-17 and BDI) were noted.

Differences in sBDNF alterations throughout a-ECT (Fig. 3 and 4) may be explained by differences in initial BDNF levels or by the small sample size of patients receiving c-ECT, limiting interpretation of our findings. Our findings also indicated that sBDNF concentrations increased abruptly after each c-ECT session (in contrast to the acute series), and decreased to initial levels within 1 month. Notable increases in BDNF after only one c-ECT may be explained by the accumulation of a BDNF pool during the former a-ECT series. It remains to be determined whether shorter c-ECT intervals lead to enduring increases in sBDNF levels, and whether such intervals provoke increases or decreases in peak BDNF levels.

Although we observed steady increases in sBDNF levels after a-ECT and c-ECT, we observed no predictive value of baseline sBDNF on depressive symptoms, nor did we observe an association between changes in sBDNF levels and depressive symptoms. There is strong evidence that antidepressant treatment reinforces the influence of environmental conditions on depression [45], and that this effect is partially facilitated by the regulatory properties of BDNF [46]. Nonetheless, previous trials examining the interplay between BDNF levels and depressive symptoms have been inconclusive [17, 22, 34, 47], and meta-analyses have reported no interaction between increased BDNF following ECT and clinical outcomes [18, 24]. Moreover, a recently published meta-analysis demonstrated that clinical responses to ECT were not associated with changes in hippocampal volume. The authors concluded that hippocampal expansion may have been caused by the therapeutic seizure itself, rather than the therapeutic effects of ECT [48]. Thus, increases in BDNF levels following ECT in the present study may represent an epiphenomenon. The impact of ECT on BDNF may be negligible or irrelevant to behavioral changes. BDNF may represent one of many components of a finely orchestrated cellular cascade, exerting only subtle effects on the clinical course. Further studies are required to determine whether ECT influences neuroplastic processes.

Several previous ECT studies measured BDNF levels in either the serum or plasma [33, 34, 36, 49-52]. In the present study, we investigated both sBDNF and pBDNF levels. Our findings indicated that sBDNF levels increased following a-ECT and c-ECT, while pBDNF levels remained unchanged. This result may be attributable to the high variation in pBDNF concentrations, suggesting that plasma is a less reliable source for measuring BDNF levels. Indeed, research has indicated that plasma assessments are more affected by operating procedures and therefore more vulnerable to error [53, 54]. The results of studies investigating both sBDNF and pBDNF levels are conflicting, and it remains to be determined which assessments of blood BDNF levels are most appropriate.

Compared to previous studies investigating the effect of ECT on BDNF levels in patients with depression, our sample size for patients receiving a-ECT is relatively large. However, the present study possesses some limitations of note. We focused on changes in BDNF levels in patients with depression receiving ECT, although we did not include a control group of patients undergoing pharmacotherapy or psychotherapy only. In addition, we did not examine other measures associated with neuroplastic processes. Included patients received psychopharmacological treatment prior to study inclusion, which can modify BDNF levels prior to a-ECT [13]. To control for this covariate, antidepressant treatment was maintained in a steady state during a-ECT, and we accounted for comedication in the statistical analysis. To yield more accurate and realistic results regarding the influence of ECT on sBDNF levels, future studies should include patients receiving ECT only and a control group undergoing psychopharmacological treatment only. However, such sophisticated study designs may lead to problems with incipient wash out from psychopharmacological treatment and selection bias in the case of the control group. In addition, we were unable to avoid decreases in sample size during follow-up investigations at V8 (n=10) and V9 (n=4) due to patient discharge. We dealt with missing data by applying a linear mixed model, which provides unbiased mean estimations for within- and between-subject effects. Nevertheless, samples V8 and V9 are relatively small, and the results should be interpreted with caution. Although approximately 1/3 of patients were switched to bilateral ECT during

the course of treatment, interaction analyses applied for potential confounders revealed no influence of these variables. We strongly challenge the possibility that increases in sBDNF levels were caused by the administration of anesthesia and muscle relaxants. Although few studies have investigated BDNF levels in patients undergoing ECT vs. sham treatment (including anesthesia and muscle relaxants), research in both humans and animals indicated that these variables do not influence or may even decrease BDNF concentrations [44, 55-58]. Less than 1/4 of patients were eligible for monthly c-ECT, and changes in antidepressant treatment are known to regularly occur after a-ECT and during c-ECT. Thus, increases in sBDNF levels after c-ECT may have been influenced by concomitant medication changes in two of these five patients. In one patient, concomitant medication was changed within the same substance class, while the other patient received augmentative therapy with agomelatine. Thus, our results regarding the effects of c-ECT should be interpreted with caution, and future studies should investigate the effects of c-ECT in larger groups of patients.

Future human *in vivo* studies may benefit from the use of combined methods to shed light on the interplay among BDNF levels, neuroplasticity, and the therapeutic efficacy of ECT in patients with depression. By integrating genetic as well as structural and functional neuroimaging modalities along with cell-molecular investigations and behavioral data, these studies may eventually be able to assess patterns of biomarker constellations. When studies focus on alterations in a single parameter, subtle changes cannot be interpreted, nor can the interplay among parameters be considered. Because we observed no correlation between sBDNF levels and therapeutic outcomes, our results suggest that sBDNF cannot be regarded as a reliable biomarker for a symptom-oriented outcome classification for individual patients.

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CONFLICT OF INTEREST

With relevance to this work there is no conflict of interest to declare. Eckert A. has received grant/research support from Schwabe, Vifor and Boiron. She has served as a consultant or on advisory boards for Vifor and Schwabe. Frey R. received speaker honoraria from AstraZeneca, Bristol-Myers Squibb, Eli Lilly and AOP Orphan. Kasper S. received grants/research support, consulting fees and/or honoraria within the last three years from Angelini, AOP Orphan Pharmaceuticals AG, Celegne GmbH, Eli Lilly, Janssen-Cilag Pharma GmbH, KRKA-Pharma, Lundbeck A/S, Mundipharma, Neuraxpharm, Pfizer, Sanofi, Schwabe, Servier, Shire, Sumitomo Dainippon Pharma Co. Ltd. and Takeda. Kranz GS received travel grants from Roche, AOP Orphan Pharmaceuticals AG and Pfizer. Lanzenberger R. received travel grants and/or conference speaker honoraria from Shire, AstraZeneca, Lundbeck A/S, Dr. Willmar Schwabe GmbH, Orphan Pharmaceuticals AG, Janssen-Cilag Pharma GmbH, and Roche Austria GmbH. Vanicek T. received travel grants and compensation for workshop participation from Pfizer and Eli Lilly and speaker honorary from Shire. Fugger G., Höflich A., Komorowski A., Milovic S., Saumer G. and Vyssoki B. declared no conflicts of interest.

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Figure 1.

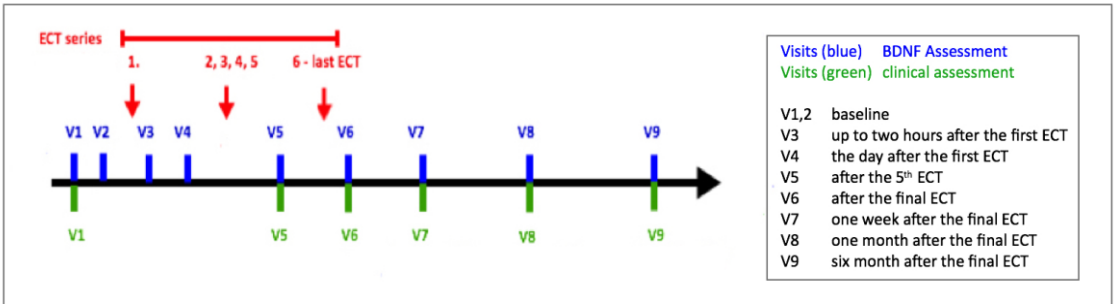

Figure 1. Study design: Longitudinal blood samples were investigated at a total of 9 visits (V) to determine serum and plasma BDNF values: V1 and V2 were baseline visits, two days and one day before the first ECT treatment; V3 was directly after the first ECT on the same day; V4 was the day after the first ECT; V5 the day after the fifth ECT; V6 the day after the last ECT; V7 one week after the last ECT; V8 one month after the last ECT and V9 six month after the last ECT. Clinical scores (HAMD-17, BPRS, BDI, MMSE) were carried out repetitively during the a-ECT at V1 and V5-V9.

Figure 2.

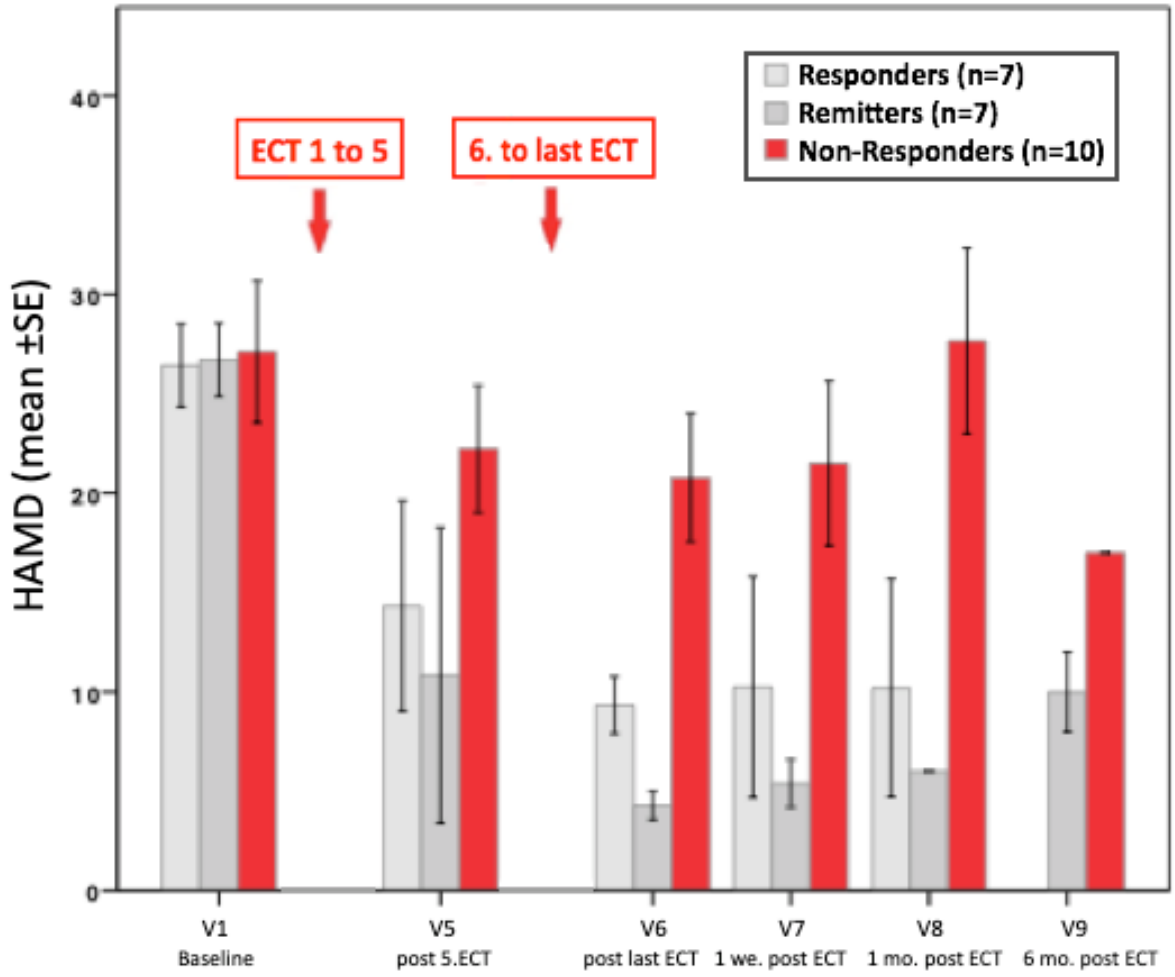

Figure 2. 17-item Hamilton Depression Rating Scale (HAMD-17) scores for remitters (dark grey, n=7), responders (light grey, n=) and non-responders (red, n=10) for acute ECT at various time-points (see x-axis). 24 patients received between 6 and 13 a-ECT, with a mean number of 9.6 (SD: ±1.7) ECTs. Treatment outcome was defined by means of HAMD at V6. HAMD scores decreased significantly following ECT ($p < 0.001$). Values are stated as mean ±SE. Post-hoc pairwise comparisons were adjusted using the Bonferroni correction for multiple comparison.

Figure 3.

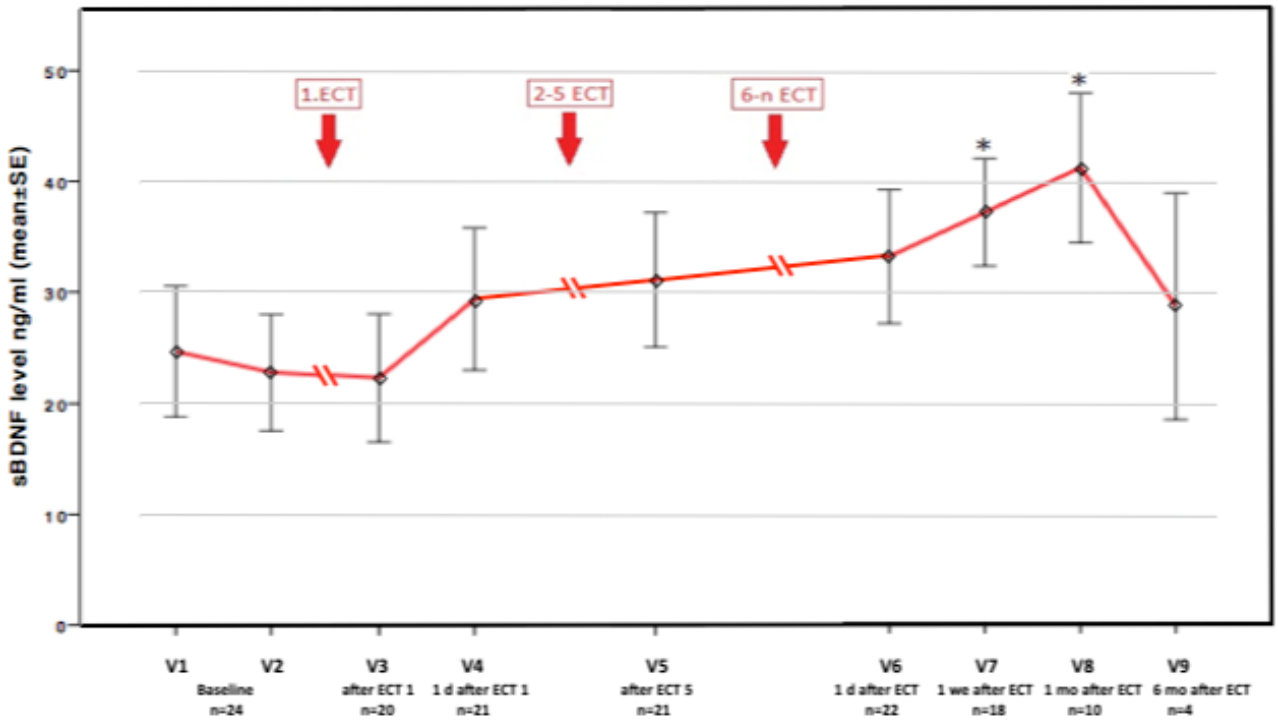

Figure 3. Serum BDNF concentrations from baseline until 6 months after the end of acute ECT in 24 patients with depression: We revealed a constant increase of sBDNF mean concentrations, starting at the day after the first ECT (V4) and continuing to one month after completion of a-ECT (V8). In comparison to baseline, we observed significantly higher sBDNF after the last a-ECT until one month after the completion of a-ECT (V6 - V8). Six months after the last a-ECT (V9), sBDNF values did not differ in comparison to baseline. Test-retest variability (V1 vs. V2) did not differ significantly. Values are stated as mean ±SE. The significance level was set at 5% in all analyses, significant differences were marked with a *. Post-hoc pairwise comparisons were adjusted using the Bonferroni correction for multiple comparison.

Figure 4

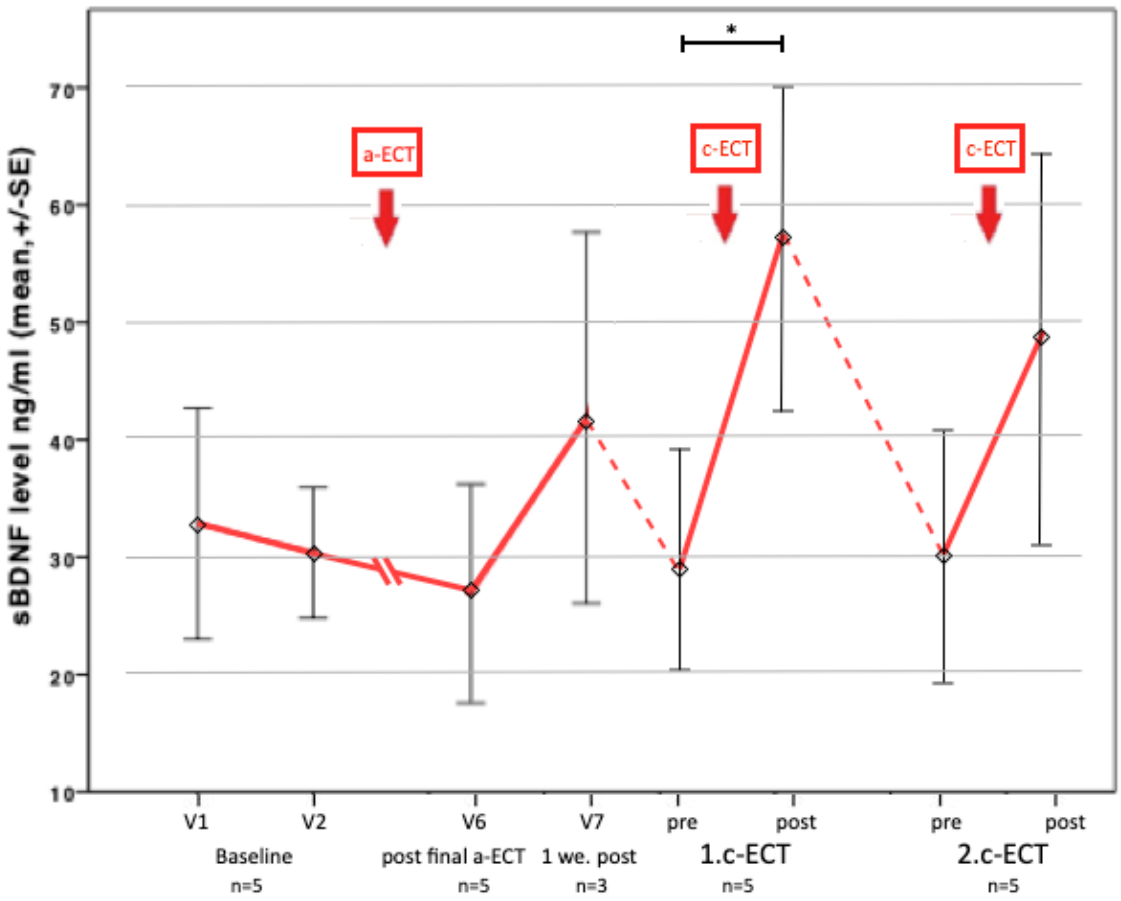

Figure 4. Serum BDNF levels are shown of five patients with major depressive disorder receiving an acute ECT series (V1 to V7, left side of the figure) and subsequent **monthly** continuation ECT (1st and 2nd c-ECT, right side of the figure). Pre and after sBDNF levels differ significantly in the first continuation ECT (c-ECT) and did not differ significantly at the second c-ECT. sBDNF values are stated as mean ±SE. The significance level was set at $p<0.05$, significant differences were marked with a *.

Table 1

ID	ICD-10 diagnosis	Age	Sex	ECT sessions	Stimulation modus	Mean seizure duration (sec; EEG)	Maximum stimulus (%)	Treatment Response
1	F33.2	36	f	11	UL/BL	57.7	60	non-responder
2	F33.2	64	f	10	UL	23.2	150	responders
3	F33.2	72	f	7	UL	22.7	200	non-responder
4	**F33.2**	**22**	**m**	**11**	**UL**	**65.3**	**50**	**non-responder**
5	**F33.2**	**70**	**f**	**9**	**UL**	**35.9**	**100**	**non-responder**
6	F33.2	52	f	13	UL	51.1	30	responders
7	F33.2	63	m	8	UL/BL	31.8	100	remitters
8	F33.2	42	f	9	UL/BL	44.6	60	non-responder
9	F33.2	70	f	10	UL/BL	54.5	100	non-responder
10	**F33.2**	**50**	**f**	**9**	**UL**	**60.2**	**50**	**remitters**
11	F33.2	34	f	9	UL	51.3	50	remitters
12	F33.3	57	m	9	UL	36.3	200	remitters
13	F33.2	73	f	12	UL	30.5	200	non-responder
14	F33.2	60	f	7	UL	57.4	60	responders
15	**F33.2**	**52**	**m**	**10**	**UL**	**59.4**	**100**	**remitters**
16	**F33.2**	**69**	**f**	**13**	**UL**	**40.5**	**200**	**responders**
17	F33.2	66	f	9	UL	36.2	180	responders
18	F33.2	49	m	6	UL	70.2	80	non-responder
19	F33.3	50	f	11	UL/BL	33.1	100	non-responder
20	F33.2	49	f	11	UL/BL	37.7	120	responders
21	F33.3	61	f	9	UL/BL	35.1	120	remitters
22	F33.2	57	m	9	UL/BL	35.1	100	non-responder
23	F33.2	58	f	10	UL	24.3	150	responders
24	F33.2	32	m	9	UL	47.1	40	remitters

Table 1. Epidemiological, ECT specific information and treatment response (according to HAMD after final a-ECT at V6) for each patient. The five patients that received c-ECT subsequent to a-ECT were marked in bold format. ECT sessions: number of a-ECT received; UL/BL: unilateral or bilateral ECT stimulation modus; mean seizure duration per person per session in seconds assessed with EEG

Table 2.

	Remitters (n=7)	Responders (n=7)	Non-Responders (n=10)
Antidepressants			
SSRI	4 Es (2), Fl (1), Se (1)	2 Es, Pa	3 Es, Fl, Se
SSNRI	1 Du	1 Du	4 Du (1), Ve (3)
SARI	1 Traz	2 Traz	-
NDRI	-	-	2 Bu
NaSSA	1 Mir	-	5 Mir
TCA	1 Mia	3 An (2), Ma (1)	3 An (2), Mil (1)
MAOI	-	3 Moc (2), Tran (1)	-
Augmentation			
Antipsychotics	7 Ar(1), Pro (1), Qu (5)	4 Am (1), Qu (2), Zi (1)	9 Am (1), Ar (1), Ol (1), Pro (1), Qu (4), Ri (1)
Mood stabil.	1 Pr	5 La (1), Pr (4)	7 Ga (1), La (2), Li (2), Pre (2)
Stimulants	-	-	1 Mod
Sedatives/Anxiolytics			
BZD	4 Lo	3 Cl (1), Lo (2)	6 Al (2), Cl (3), Lo (1)
Z-substances	1 Zo	1 Zo	-

Table 2. Concomitant medication used for patients within the a-ECT series and listed according to outcome. Patients who responded to treatment exhibited a 50% decrease in HAMD scores after the last ECT (at V6). Remission was defined by a HAMD score below seven. Numbers in brackets indicate the proportion of each concomitant medication taken by a patient. SSRI: Selecitvie noradrenaline reuptake inhibitors; SNRI: Serotonin–norepinephrine reuptake inhibitors, SARI: Serotonin antagonist and reuptake inhibitors, NaSSA: Noradrenergic and Specific Serotonergic Antidepressant, NDRI: Norepinephrine-dopamine reuptake inhibitor, TCA: Tricyclic antidepressant, MAOI: Monoamine oxidases inhibitors, BZD: Benzodiazepines; Al: Alprazolam, Am: Amisulpride, An: Anafranile, Ar: Aripiprazole, Bu: Buporpion, Cl: Clonazepam, Du: Duloxetine, Es: Escitaloprame, Fl: Fluoxetine, Ga: Gabapentin, Li: Lithium, La: Lamotrignie, Lo: Lorazepam, Ma: Maprotiline, Mia: Mianserin, Mil: Milnacipran, Mir: Mirtazapine, Moc: Moclobemid, Mod: Modafinile, Ol: Olanzapine, Pa: Paroxetine, Pre: Pregabalin, Pro: Prothipendyl, Qu: Quetiapine, Ri: Risperidone, Se: Sertraline, Tran: Tranylcypromin, Traz: Trazodone, Ve: Venlafaxin, Zi: Ziprasidone, Zo: Zolpidem.

Table 3.

Visit	1		2		3		4		5		6		7		8		9	
	n	mean ±SD	n	mean ±SD	n	mean ±SD	n	mean ±SD	n	mean ±SD	n	mean ±SD	n	mean ±SD	n	mean ±SD	n	mean ±SD
sBDNF (ng/ml)	24	24.68 ±14.40	22	22.79 ±12.30	20	22.3 ±12.91	21	29.4 ±14.71	21	31.14 ±13.90	22	33.04* ±14.11	18	37.03* ±10.29	10	41.05* ±10.67	4	28.56 ±10.19
pBDNF (pg/ml)	24	0.76 ±1.46	22	0.81 ±1.6	20	0.89 ±1.8	22	1.19 ±2.25	22	0.84 ±1.8	20	0.88 ±1.67	17	1.25 ±2.36	11	0.74 ±1.66	4	0.3 ±0.28
HAMD	24	26.79 ±4.01							21	16.71* ±8.13	22	12.41* ±8.04	18	14.72* ±8.84	10	14.60* ±10.22	4	13.50* ±4.12
BPRS	22	48.77 ±14.25							19	35.63* ±10.44	20	32.35* ±10.98	18	35.17* ±14.33	8	36.63* ±17.61	1	38*
BDI	21	32.38 ±9.60							19	21.05* ±12.59	20	15.75* ±13.23	17	19.65* ±14.31	8	22.00* ±16.71	1	31
MMSE	22	28.14 ±2.05							19	28.53 ±1.54	20	28.25 ±1.86	18	28.27 ±1.48	8	28.38 ±2.07	1	29

Table 3. Mean values and standard deviation (SD) and number of patients for sBDNF (in ng/ml), pBDNF and psychometric scores (HAMD, BPRS, BDI, MMSE) for each time-point before, during and after acute ECT. Description of those patients with HAMD and sBDNF values available at different time points. Time schedule for Visits 1-9 (V1-9) is defined as follows: V1 and V2: baseline (test-retest); V3: up to two hours after the first ECT; V4: the day after the first ECT; V5: the day after the fifth ECT; V6: the day after the final ECT; V7: one week; V8: one month; V9: six months after the final ECT. *denotes a significant difference to Visit 1 at a corrected $p<0.05$ using post-hoc t-tests and using Wilcoxon signed-rank test, respectively. sBDNF: serum Brain Derived Neurotropic Factor, pBDNF: plasma Brain Derived Neurotropic Factor, HAMD: Hamilton Depression Rating Scale, BPRS: Brief Psychiatric Rating Scale, BDI: Beck-Depression-Inventar, MMSE: Minimental State Examination

Table 4.

Visit / sBDNF	1	2	6	7
Remitters	27.0 ±15	24.7 ±12.14	29.84 ±12.96	40.57 ±11.30
Responders	29.21 ±17.14	26.59 ±12.8	35,82 ±21.52	34.96 ±11.39
Non-responders	19.88 ±11.78	17.8 ±11.84	33.69 ±9.6	35.98 ±10.07

Table 4. Mean values and standard deviation (SD) of sBDNF in ng/ml at baseline (Visit 1 and 2) as well as one day and one week after the final acute ECT (V6 and V7). Serum BDNF concentrations are stated according to outcome of acute ECT, for remitters, responders and non-responders. Treatment outcome was defined by means of HAMD at V6.